CORRESPONDENCE

Re: Human Papillomavirus in Oral Exfoliated Cells and Risk of Head and Neck Cancer

I read with interest the article by Smith et al. *(1)* reporting an association between human papillomavirus (HPV) and head and neck squamous-cell carcinoma (HNSCC). Their study is consistent with two previous reports of the association of HPV with HNSCCs and especially with tonsillar and oropharyngeal cancers *(2,3)*. These three studies suggest that approximately 25% of tonsillar and oropharyngeal cancers are linked with HPV-induced carcinogenesis. In addition, all three studies found a preponderance (i.e., 85% or more) of HNSCCs with HPV type 16 (HPV16) DNA, the type that causes approximately 50% of cervical cancers. These data are consistent with an etiologic role for HPV in the development of a subset of HNSCCs and the notion that HPV16 is uniquely oncogenic.

However, Smith et al. *(1)* state that "HPV testing of an oral rinse may be predictive of HPV-related head and neck cancers." Such a statement warrants caution. Although oral HPV DNA was strongly associated with having HNSCC compared with HPV DNA–negative control subjects (odds ratio [OR] = 11.5, 95% confidence interval [CI] = 5.2 to 25.7), the association was substantially less than the more than 100-fold (odds ratio) associations between HPV DNA in exfoliated cervical cells and cervical cancer. This order-of-magnitude difference in strength of the association is partly the result of the non-HPV etiology of HNSCCs but also of the weak concordance between oral HPV status and HPV positivity of the tumor (κ = 0.40, 95% CI = 0.23 to 0.57). Oral HPV testing by Smith et al. *(1)* was more accurate for that detection of HNSCC than that previously observed *(2)*. Combining data from these two studies (Table 1), κ was 0.33 (95% CI = 0.18 to 0.47). Unlike HPV testing of exfoliated cervical specimens that can be highly concordant with biopsy HPV DNA (due to the proximity of the sampling to the tumor), oral HPV DNA status may reflect an exposure to HPV that is often unrelated to having a cancer in other anatomic locations of the head and neck. A recent study found that the prevalence of HPV DNA in oral rinses was significantly higher than that in transepithelial brush biopsy specimens of the tonsillar region *(4)*, suggesting that oral HPV DNA status poorly reflects HPV status elsewhere. Thus, the combination of high prevalence of oral HPV DNA in non-case subjects and poor agreement of oral HPV DNA status in case subjects will limit the usefulness of oral HPV DNA testing for HPV-related HNSCCs, except possibly among patients, such as Fanconi anemia patients, who have a predilection for these malignancies and who are uniquely susceptible to HPV-induced carcinogenesis and are at a high risk of an HPV16-induced HNSCC *(5)*.

Table 1. Cross-tabulation of oral human papillomavirus (HPV) DNA status and HPV DNA status of tumor biopsy specimens from patients with head and neck squamous-cell carcinoma*

	HPV DNA in oral cells		
HPV DNA in tumor	No. negative (%)	No. positive (%)	Total
No. negative (%)	443 (93.5) (129 + 314)	31 (6.5) (16 + 15)	474 (145 + 329)
No. positive (%)	41 (63.1) (23 + 18)	24 (36.9) (22 + 2)	65 (45 + 20)
Total	484 (152 + 332)	55 (38 + 17)	539 (190 + 349)

*Number pairs in parentheses represent the respective contributions from Smith et al. *(1)* and Herrero et al. *(2)*, respectively. Row percentages are provided (for all data, κ = 0.33 [95% confidence interval {CI} = 0.18 to 0.47]; 86.6% agreement [95% CI = 83.5% to 89.4%] and 25.0% positive agreement [95% CI = 0.18 to 0.47]; relative sensitivity = 36.9% [95% CI = 25.3% to 49.8%]; relative specificity = 93.5% [95% CI = 90.9% to 95.5%]).

Other biomarkers (e.g., $p16^{INK4A}$ expression or 3q amplification) could be considered for molecular diagnostics for detection of HNSCC, but like HPV DNA, these may require more invasive cell collections from the tonsils and/or oropharynx than simple oral collections for diagnostic accuracy. Serum assays for HPV16 oncoproteins E6 and E7 are highly specific for HNSCC and better correlate with HPV DNA status of the tumor than oral HPV DNA status but may not be sufficiently sensitive *(2)*. Promising new serum-based proteomic assays for detection of HNSCC *(6)* await further validation.

Finally, although screening and/or early detection of HPV16-induced HNSCC may remain intractable for the near future, this cancer may be preventable by vaccination with HPV16 L1 virus–like particle vaccines, which have shown 100% prophylactic efficacy for cervical HPV16 infections *(7)*. Whether these vaccines will similarly prevent HPV16 infections of the head and neck remains unknown. Targeted vaccination of high-risk populations such as Fanconi anemia patients may address the prophylactic potential of these vaccines for preventing HPV infections of the head and neck, confirm the causal link between HPV16 infection and HNSCC, and provide a much needed intervention for these patients.

PHILIP E. CASTLE

REFERENCES

(1) Smith EM, Ritchie JM, Summersgill KF, Hoffman HT, Wang DH, Haugen TH, et al. Human papillomavirus in oral exfoliated cells and risk of head and neck cancer. J Natl Cancer Inst 2004;96:449–55.

(2) Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003;95:1772–83.

(3) Gillison ML, Shah KV. Human papillomavirus-associated head and neck squamous cell carcinoma: mounting evidence for an etiologic role for human papillomavirus in a subset of head and neck cancers. Curr Opin Oncol 2001;13:183–8.

(4) Kreimer AR, Alberg AJ, Daniel R, Gravitt PE, Viscidi R, Garrett ES, et al. Oral human papillomavirus infection in adults is associated with sexual behavior and HIV serostatus. J Infect Dis 2004;189:686–98.

(5) Kutler DI, Wreesmann VB, Goberdhan A, Ben Porat L, Satagopan J, Ngai I, et al. Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. J Natl Cancer Inst 2003;95:1718–21.

(6) Wadsworth JT, Somers KD, Cazares LH, Malik G, Adam BL, Stack BC Jr, et al. Serum

protein profiles to identify head and neck cancer. Clin Cancer Res 2004;10:1625–32.
(7) Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002;347:1645–51.

NOTES

Correspondence to: Philip E. Castle, PhD, MPH, Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, 6120 Executive Blvd., MSC 7234, Bethesda, MD 20892-7234 (e-mail: castlep@mail.nih.gov).

DOI: 10.1093/jnci/djh232

RESPONSE

Dr. Castle's caution regarding human papillomavirus high-risk (HPV-HR) types in head and neck cancer is, in our opinion, unduly pessimistic. Although he recognizes that our oral HPV testing was more accurate for the detection of head and neck cancer (HNC), he lumps our results *(1)* with those of a study by the IARC consortium *(2)* that reported lower HPV-HR prevalence in case and control subjects alike. We believe that combining the results (his table 1) is misleading in its interpretation because the IARC study concerned a different population and used different specimen collection and HPV evaluation methods. We overcame some of the limitations of HPV detection with improved sample collection and by monitoring the nucleated cell count in each cytology specimen with a hemocytometer. Consequently, 99.1% of our oral rinses were adequate for PCR compared with 43.6% in *(2)*.

HPV-HR–associated cancers and HPV-HR infection in the head and neck area in general are more common than the 25% HPV-positive tonsillar and oropharyngeal cancers stated by Dr. Castle. Whereas the IARC study *(2)* reported 18% HPV-HR–positive HNCs in these sites, we *(1)* and Gillison and Shah *(3)* reported 38% and 57%, respectively. We identified HPV-HR–positive tumors among non–squamous-cell histologic types of HNC and at oral cavity sites other than the tonsils or the oropharynx *(1)*. Association with HPV-HR infection is therefore not limited to squamous-cell carcinoma or to tumor sites in the tonsillar area or oropharynx. We believe that an oral rinse has a greater potential for including HPV-HR–infected cells from the entire oral cavity and oropharynx than targeted scrapes or biopsies that can examine only localized areas and would miss a neighboring focus of HPV-infected or HPV-positive cancer cells. Our experience is in agreement with the findings of Lawton et al. *(5)*, who reported consistent cytology specimen collection using oral rinses.

Although HPV type 16 (HPV16) is the most common HPV-HR type in HNC, it is not unique: We and others *(1–4)* have identified other cervical cancer–causing HPV-HR types in HNC. Indeed, we used laser-directed microdissection to isolate the tumor tissue and confirmed, by DNA sequencing, the HPV type in tumors that were located outside of the tonsils or oropharynx and in those harboring a virus type other than HPV16 *(1)*. It is apparent that HPV16 is more common in HPV-positive cancers in the head and neck (up to 85%) than in the cervix (~50%). It will be interesting to determine whether keratinocytes from the oral cavity, tonsillar fossa, or oropharynx exhibit different susceptibilities to infection with and establishment of persistence by different HPV-HR types.

Finally, Dr. Castle raises conceptual concerns regarding HPV detection as a biomarker of HPV-associated HNC. He states that, compared with cervical cancer, the strength of the association between HPV-HR infection and HPV-HR–positive HNC is weakened by a lower concordance between oral HPV status and HPV positivity of the tumor. We discussed possible limitations of HPV-HR detection in oral rinses *(1)*. HPV-HR DNA in oral cytology specimens may come from HPV-positive cancer cells, cells from the HPV-infected field in which the HPV-associated tumor arose, or from an independent infection with the same or another HPV-HR type. Nevertheless, the detection of even an unrelated HPV-HR type in the oral cavity may identify individuals who are more susceptible to infection because of their limited immunologic ability to eliminate the virus or other unknown causes. This approach thus broadens the captured group of individuals at risk and would be predicted to encompass those with Fanconi anemia *(6,7)*, as Dr. Castle suggests, or possibly with other diseases affecting genome maintenance. We disagree that this decreases the value of HPV-HR detection as a potential biomarker for HPV-associated HNC. Clearly, most cigarette smokers do not develop lung cancer, yet smoking is a significant risk factor and predictor of those who are more likely to develop lung cancer in their lifetimes. Likewise, we argue that, on the basis of our findings, people with a detectable HPV-HR–type infection may be at an increased risk of developing an HPV-HR–associated HNC, a deadly disease for which no screening or early diagnostic tests are currently available. Thus, we believe that data from our laboratory and others warrant further exploration of HPV detection as a biomarker for the identification of individuals who are at risk of developing an HPV-associated cancer of the head and neck.

ELAINE M. SMITH
THOMAS H. HAUGEN
LUBOMIR P. TUREK

REFERENCES

(1) Smith EM, Ritchie JM, Summersgill KF, Hoffman HT, Wang DH, Haugen TH, et al. Human papillomavirus in oral exfoliated cells and risk of head and neck cancer. J Natl Cancer Inst 2004;96:449–55.
(2) Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003;95:1772–83.
(3) Gillison ML, Shah KV. Human papillomavirus-associated head and neck squamous cell carcinoma: mounting evidence for an etiologic role for human papillomavirus in a subset of head and neck cancers. Curr Opin Oncol 2001;13:183–8.
(4) Schwartz SM, Daling JR, Doody DR, Wipf GC, Carter JJ, Madeleine MM, et al. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. J Natl Cancer Inst 1998;90:1626–36.
(5) Lawton G, Thomas S, Schonrock J, Monsour F, Frazer I. Human papillomaviruses in normal oral mucosa: a comparison of methods for sample collection. J Oral Pathol Med 1992;21:265–9.
(6) Lowy DR, Gillison ML. A new link between Fanconi anemia and human papillomavirus-associated malignancies. J Natl Cancer Inst 2003;95:1648–50.
(7) Kutler DI, Wreesmann VB, Goverdhan A, Ben-Porat L, Satagonpan J, Ngai I, et al. Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. J Natl Cancer Inst 2003;95:1718–21.

NOTES

Affiliation of authors: Department of Epidemiology, College of Public Health (EMS), VAMC and Department of Pathology, Carver College of Medicine (THH, LPT), University of Iowa, Iowa City.

Correspondence to: Elaine M. Smith, PhD, MPH, Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA 52242 (e-mail: elaine-smith@uiowa.edu).

DOI: 10.1093/jnci/djh233